

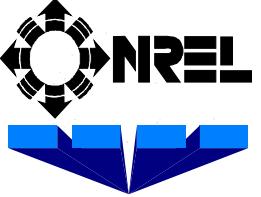
# *Zymomonas mobilis*

**Special Topics Session  
Microbial Pentose Metabolism**

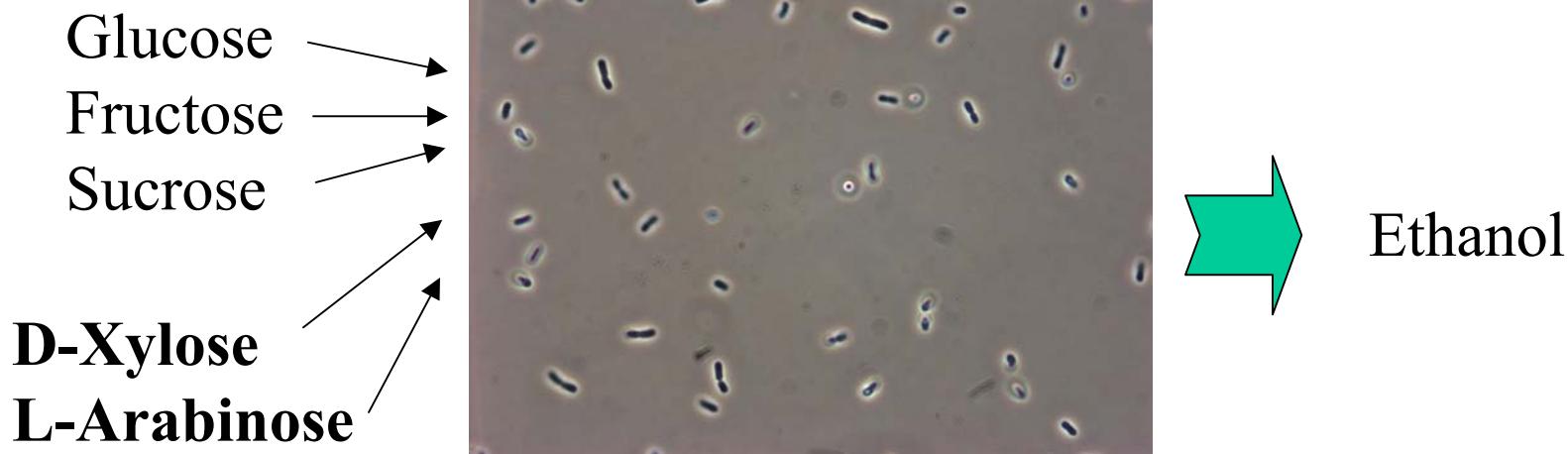
**25th Symposium on  
Biotechnology for Fuels and Chemicals**

May 5, 2003  
Min Zhang  
National Bioenergy Center  
National Renewable Energy Laboratory



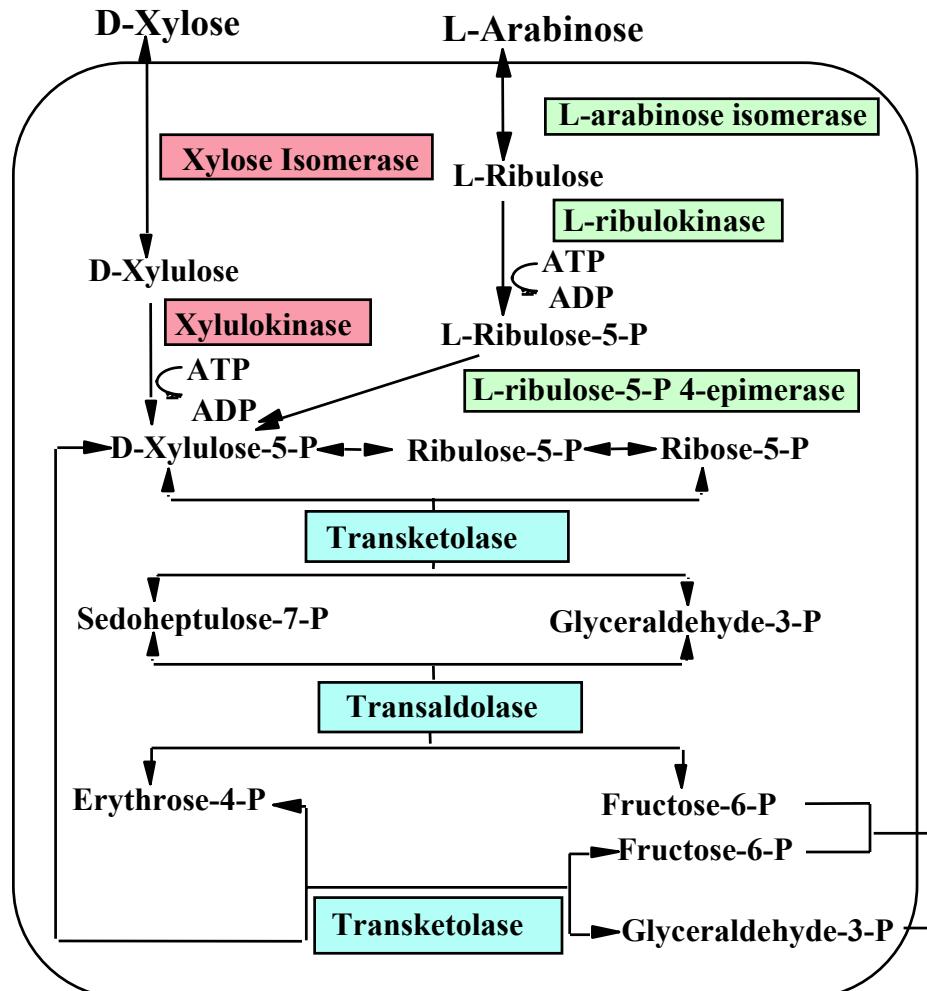


# *Zymomonas mobilis*

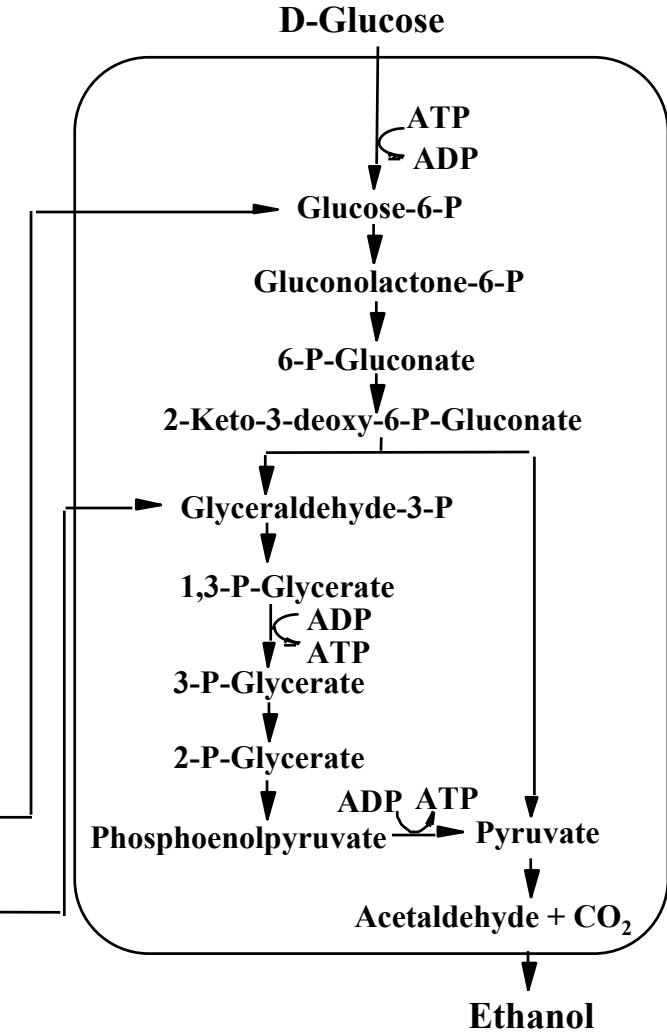


# Metabolic Engineering for Pentose Fermentation

## Pentose Metabolism Pathway



## Entner-Doudoroff Pathway





# r*Zymomonas* Strains Developed

- **Xylose-Fermenting Strains**
  - Host CP4: CP4(pZB4) and CP4(pZB5) etc.
  - Host 39676: 39676(pZB4), 39676(pZB5) and 39676(pZB4L)
    - Hydrolysate-adapted 39676(pZB4L)
    - C25 (genomic integrated)
  - Host 31821/ZM4: ZM4(pZB5)
    - 321(5), 2032 and 8b (genomic integrated)
- **Xylose and Arabinose-Fermenting Strains**
  - Host 39676: 206C(pZB301) and 206C(pZB401)
    - BC1(pZB301)
    - AX strains (genomic integrated)
- **Xylose, Arabinose and Mannose-Fermenting Strains**



## Highlights of *Zymomonas mobilis*

- Natural fermentative microorganism (GRAS)
- High ethanol yield from glucose (95-98% or 0.49-0.50 g/g)
- Low cell mass formation
- No oxygen requirement
- High ethanol tolerance (13% ethanol from 30% glucose)
- High specific productivity ( $2\text{-}6 \text{ g ethanol g dcw}^{-1} \cdot \text{hr}^{-1}$ )
- High sugar uptake rate (up to  $10 \text{ g glucose g dcw}^{-1} \cdot \text{hr}^{-1}$ )

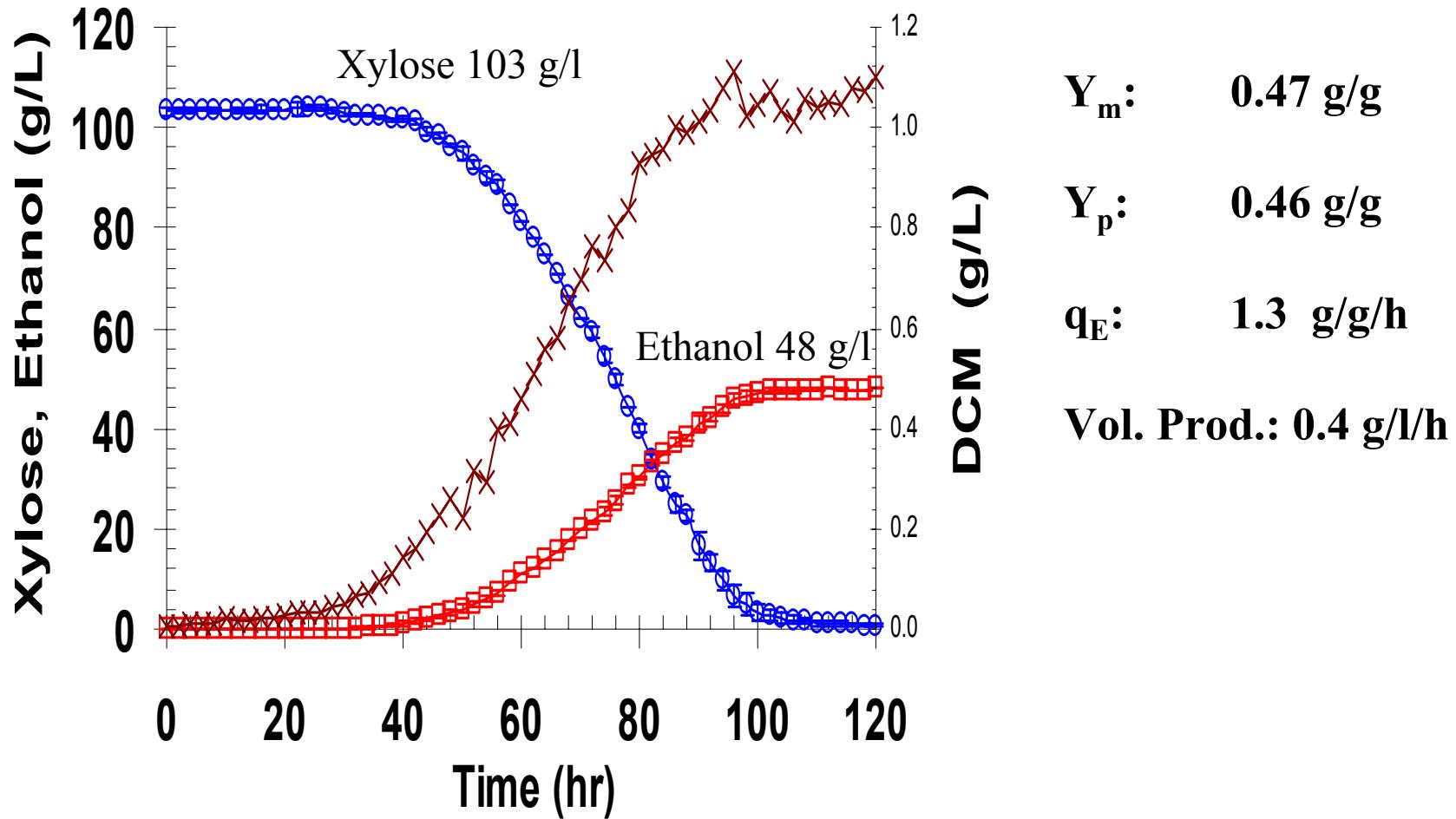


# Cultivation Conditions

- Media:
  - RM: 10 g/L YE, 2 g/L KH<sub>2</sub>PO<sub>4</sub>, 2% glucose (desired sugars)  
or  
Clarified CSL (cCSL) (1%) + sugars
  - Fermentation Media using hydrolysate : cCSL (1%) + Hydrolysate, overlimed or neutralized supplemented with extra glucose.
- Inoculum Size: 0.03-0.25 OD @600 nm (0.01 - 0.085 g cell/L)
- Fermentor: pH 5 or pH 6 controlled with KOH (2N)
- Temperature at 30°C or 37°C
- No aeration

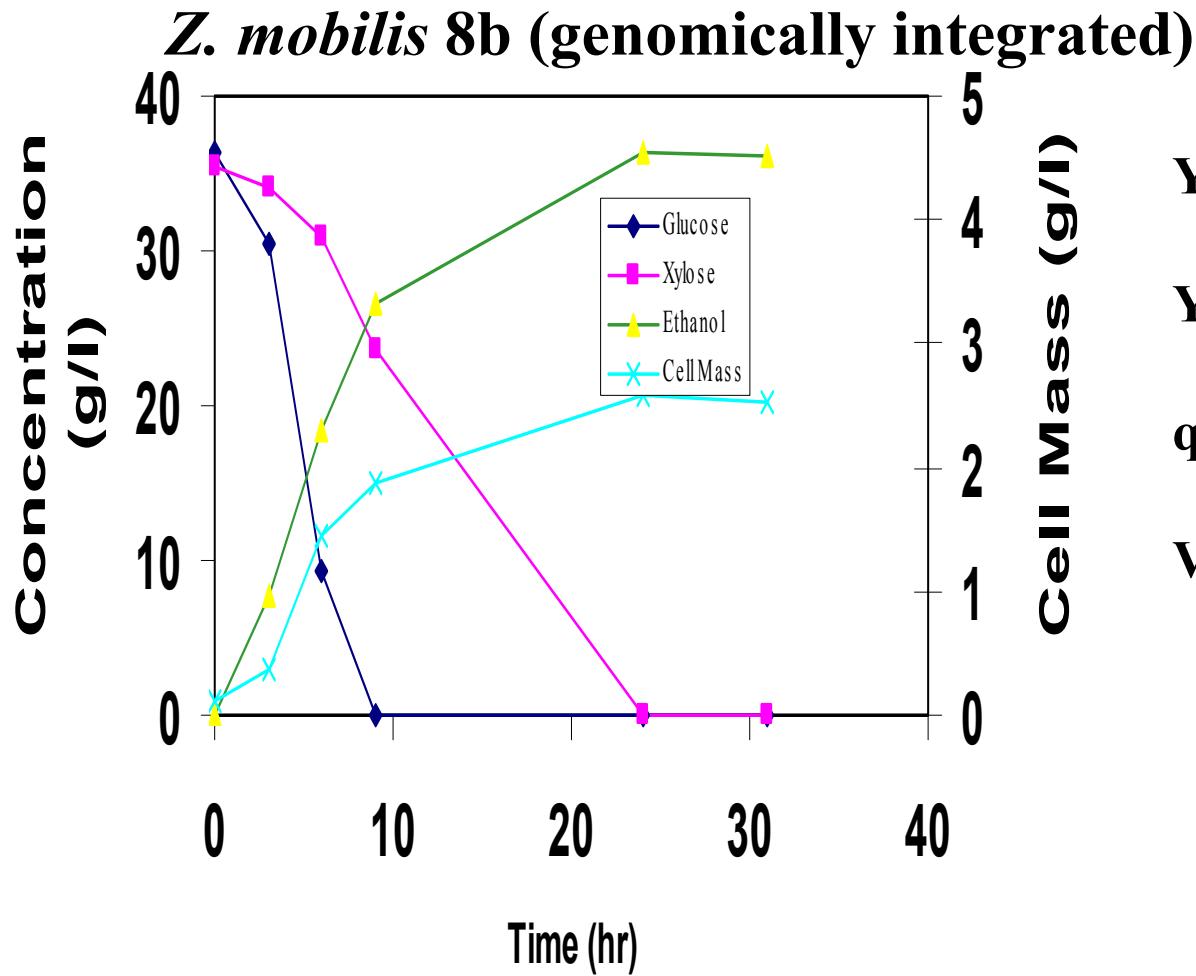


# *Z. mobilis* 8b on RM 10% xylose





# Fermentation of Xylose-Fermenting *Z. mobilis* Strain



$Y_m:$  0.51 g/g

$Y_p:$  0.51 g/g

$q_E:$  1.13 g/g/h

Vol. Prod.: 1.5 g/l/h

RM media, pH=5.5, T=30°C



# Corn Stover Hydrolysates

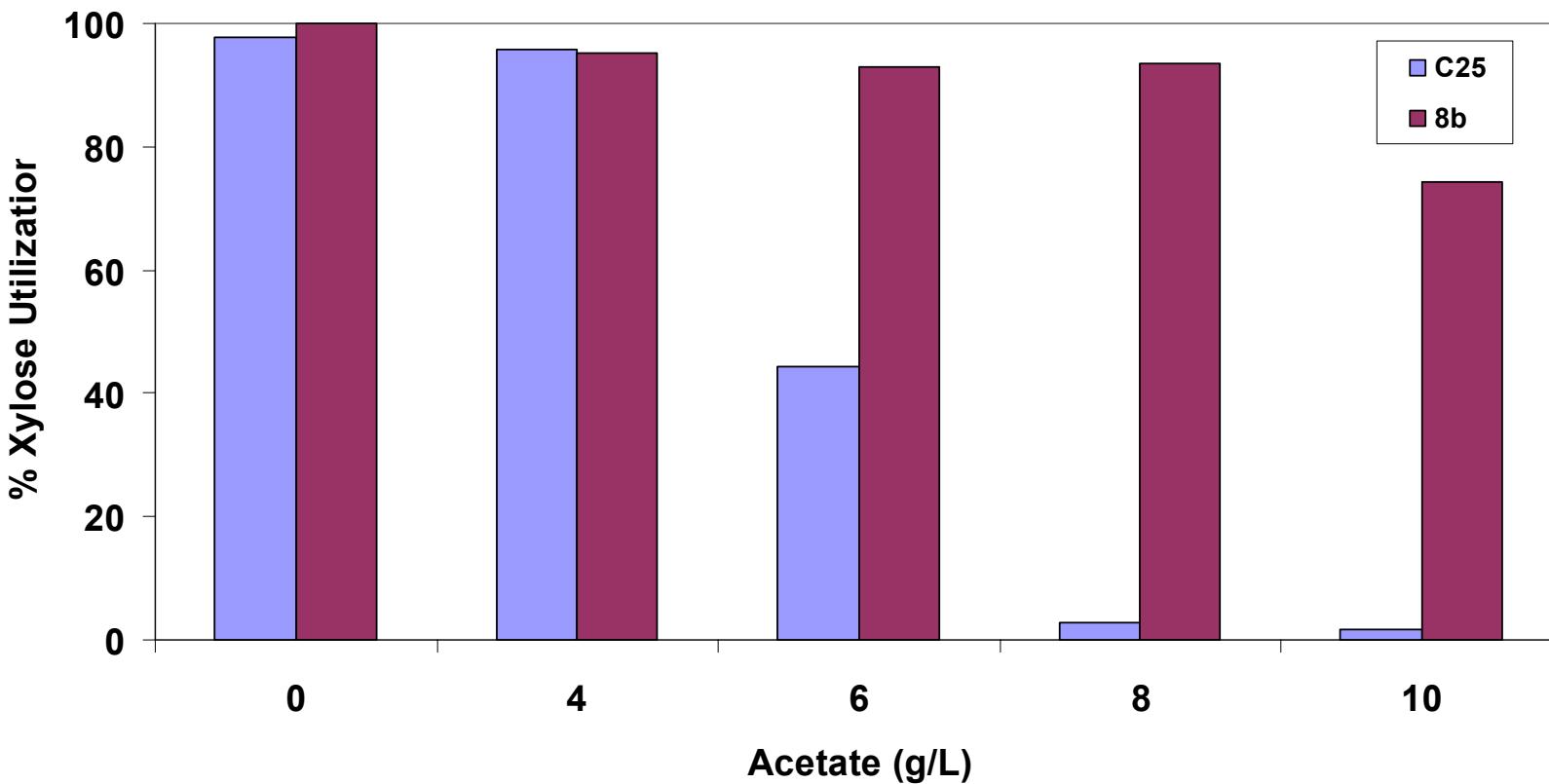
Compound	Concentration (g/L)	Total Sugar Conc. (g/L)
Cellobiose	1.91	110
Glucose	15.65	
Xylose	69.20	
Arabinose	11.87	
Galactose	6.63	
Mannose	5.05	
Acetic acid	10.98	
Lactic acid	2.15	
HMF	0.67	
Furfural	1.14	
Acid soluble lignin	10.77	

Pretreatment: using the flow-through mode of operation and at 25% solids, 190°C and 0.048 g acid/g dry biomass (Schell et al. 2002).

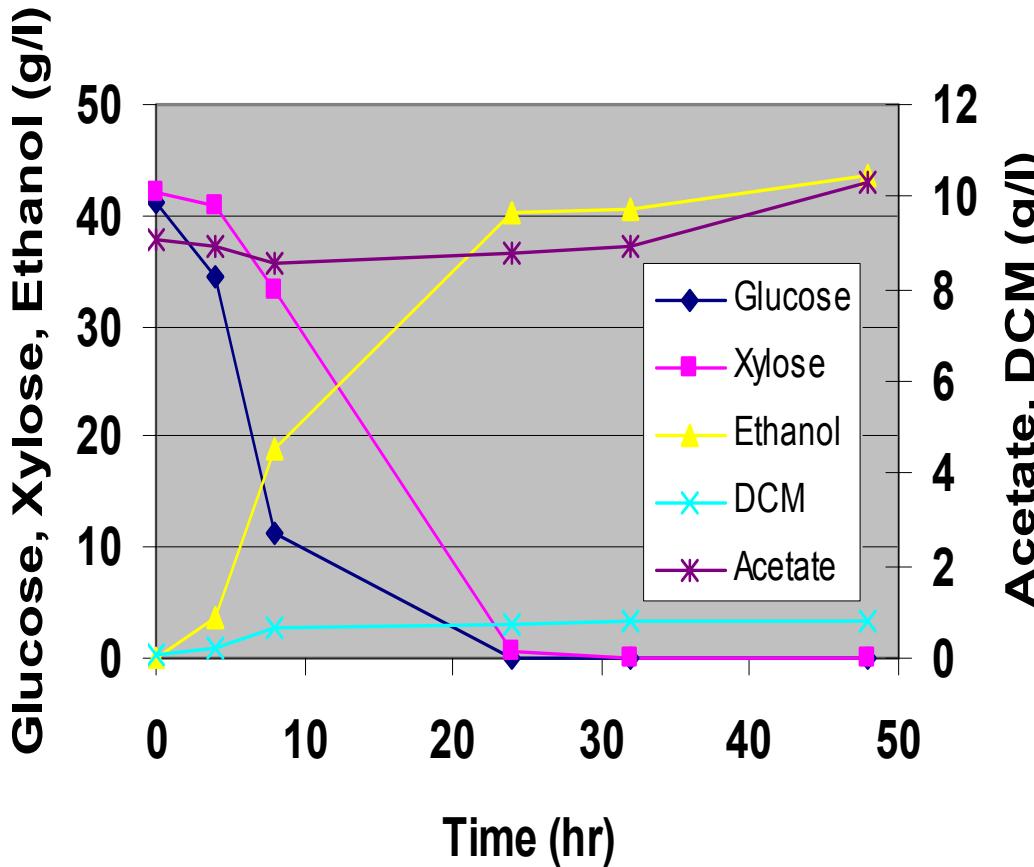


# Increased Acetate Tolerance

**Comparison of %Xylose Utilization by Strains C25 and 8b  
at T 37°C, Grown on RMGX (2%:2%) in Baffled Shake Flask**



# Fermentation of Strain *Z. mobilis* 8b in cCSL+4% glu + 4 % xyl in the presence of 8 g/l Acetate at 37 C at pH 6



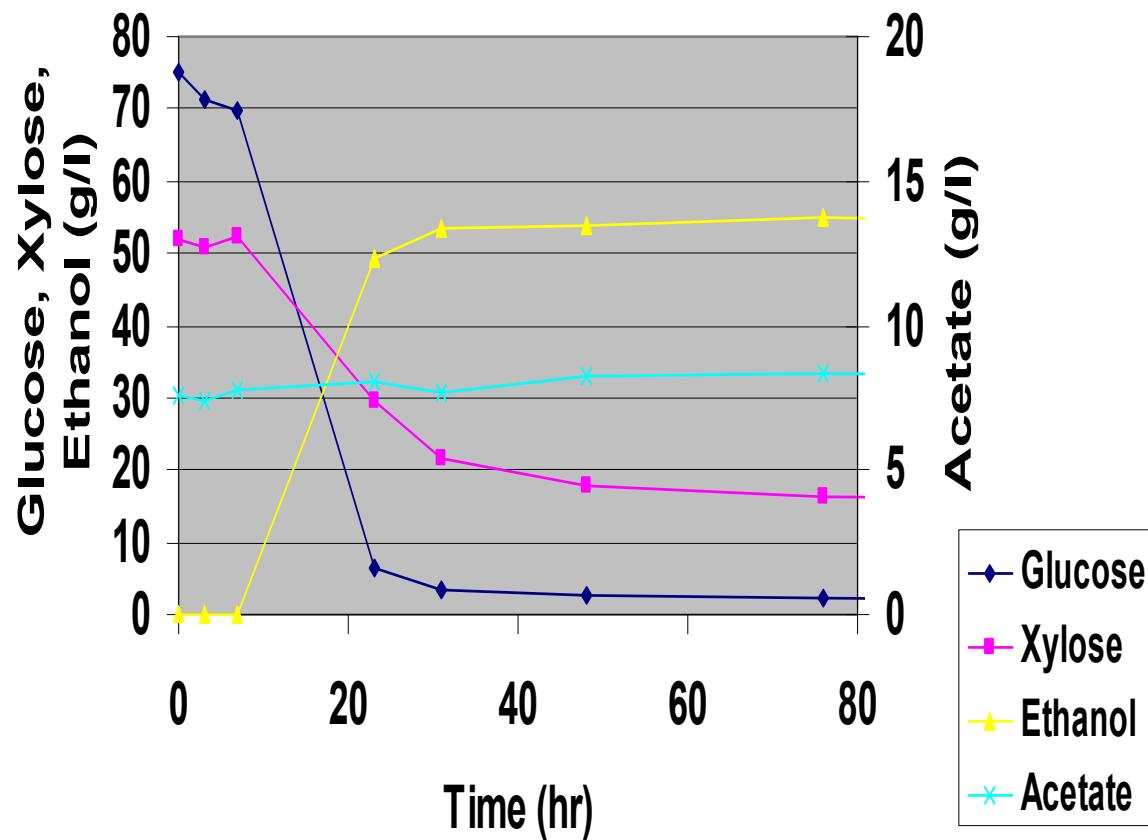
$Y_m$ : 0.52 g/g

$Y_p$ : 0.52 g/g

$q_E$ : 4.1 g/g/h

Vol. Prod.: 1.68 g/l/h

## Fermentation profile of *Z. mobilis* 8b in 80% OL-Corn Stover Hydrolysate spiked with glucose at pH6 and 37C



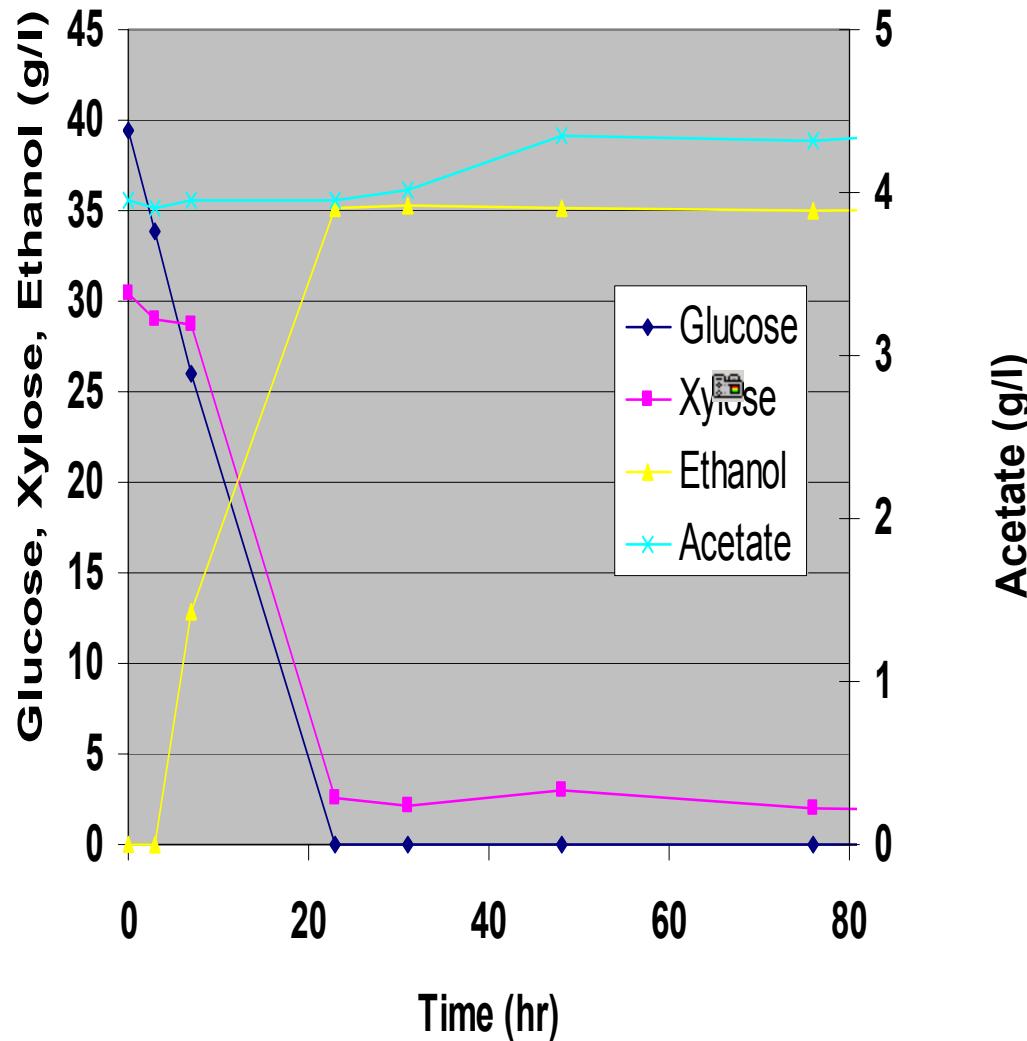
$Y_m$ : 0.52 g/g

$Y_p$ : 0.42 g/g

Vol. Prod.: 2.1 g/l/h



## Fermentation of *Z. mobilis* 8b in 40% Neutralized CSH spiked with glucose at 37 C pH6



$Y_m:$  **0.52 g/g**

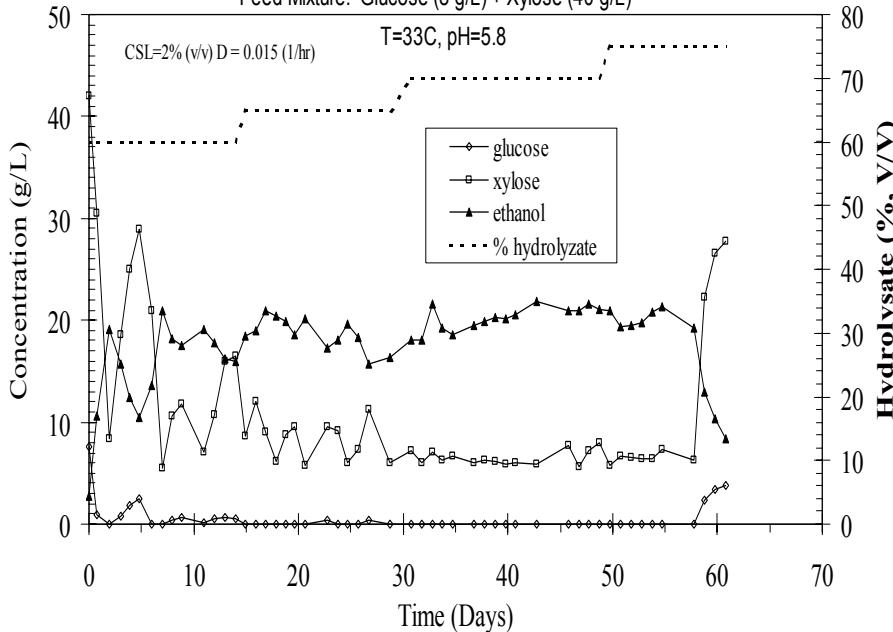
$Y_p:$  **0.51 g/g**

**Vol. Prod.: 1.45 g/l/h**

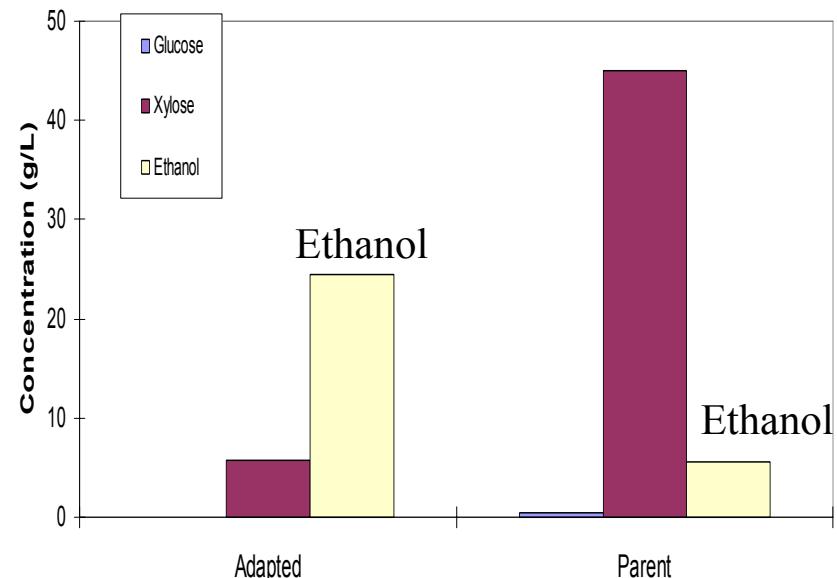


# Strain Improvement through Adaptation

Adaptation of *Zymomonas* 39676(pZB4L) to above 60% (v/v) OL Hydrolyzate  
Feed Mixture: Glucose (8 g/L) + Xylose (40 g/L)



Comparison of Adapted and Parent Strain 39676(pZB4L) Grown on cCSL (2%),  
60% Sawdust Hydrolysate at pH 5.8, T 30°C, Initial Sugar Conc. (G:X 1%:5%)





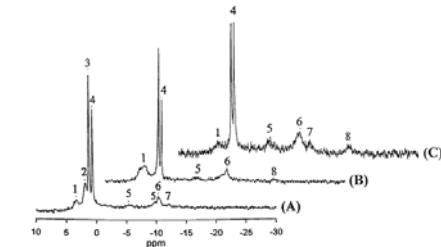
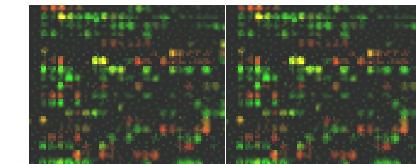
# Future Improvements

- Ferment all biomass sugars (including mannose and galactose)
- Improve pentose fermentation yield and productivity
- Improve tolerance to hydrolysate toxicity

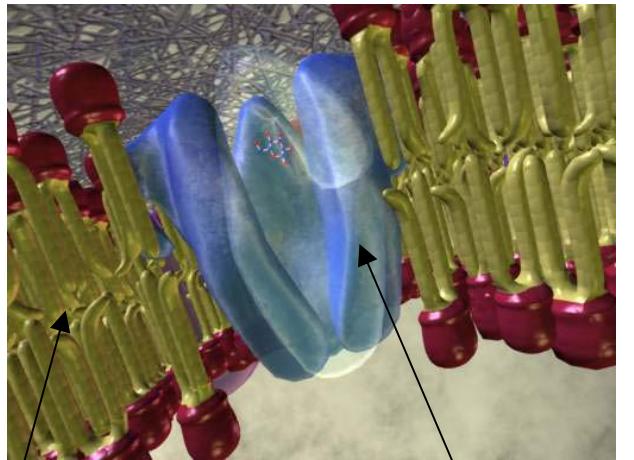


# Metabolic Engineering Tools

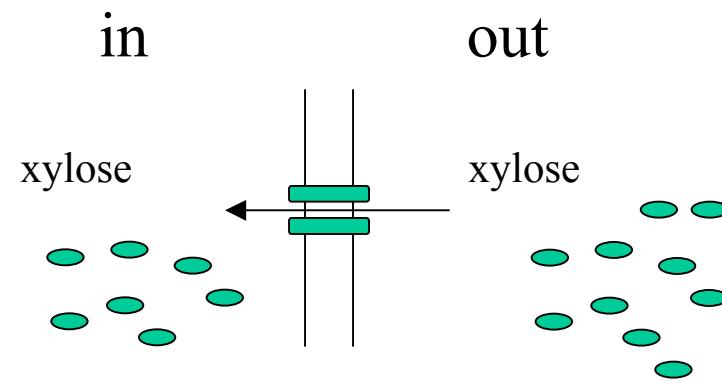
- Transcriptomics
  - Examine gene expression at global level
- Metabolomics
  - Examine metabolites at global level
- High-throughput Enzyme Assays
  - Compare strains
  - Compare activities at different stages of growth
  - Compare activities in different growth conditions
  - Develop improved enzymes
- Mathematical approaches
  - Metabolic flux analysis
  - Kinetic modeling



# Is Sugar Transport limiting?



Cytoplasmic membrane  
Transporter protein



Facilitated Diffusion Transport

No energy required



# Acknowledgements

**The Office of the Biomass Program  
of the US Department of Energy**